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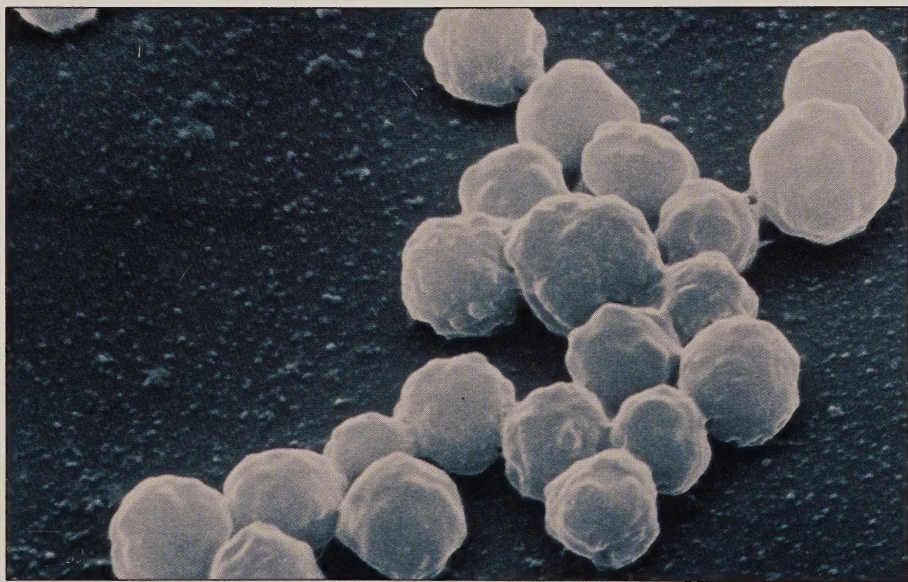
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GYPCHEK

The Gypsy Moth Nucleopolyhedrosis Virus Product

Richard Reardon
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Richard Reardon¹ and John Podgwaite²

The gypsy moth, *Lymantria dispar* Linnaeus, is a serious defoliator of broadleaved forests in eastern North America. Populations of this insect pest undergo periodic outbreaks to extremely high densities that result in widespread defoliation to an average of 5.0 million forested acres per year. In addition, this pest defoliates trees and shrubs in residential areas and when infestations are heavy, creates a nuisance to residents. Since the introduction of the gypsy moth and its associated infectious diseases into the Boston area in 1869, it has spread to the south and west and continues to spread at the rate of approximately twelve miles per year (Figure 1).

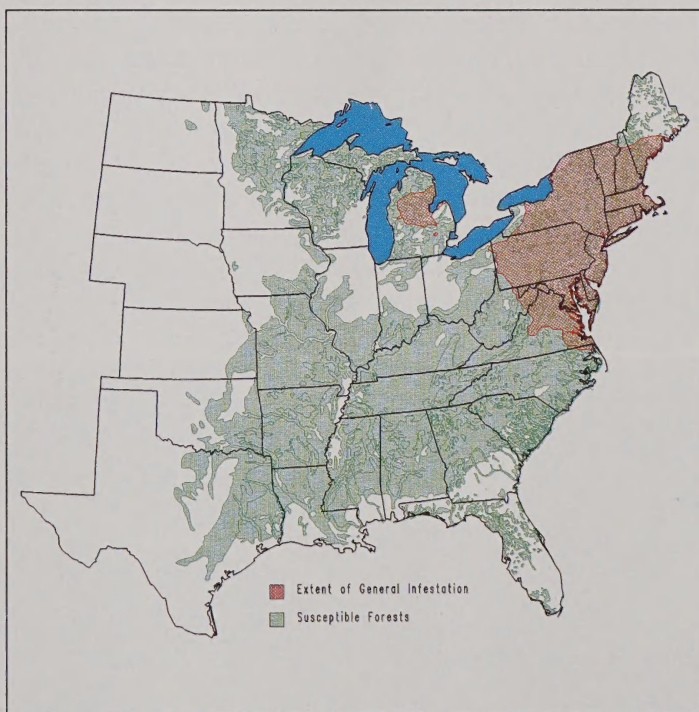


Figure 1. Distribution of the Gypsy Moth and Susceptible Oak-Type Forests of the Eastern United States

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In eastern North America, the gypsy moth is subject to a variety of naturally occurring infectious diseases caused by several kinds of bacteria and fungi as well as a virus (Podgwaite and Campbell 1972). The virus is a member of the genus *Baculovirus* and is totally unrelated to viruses that cause disease in humans and other animals. The naturally occurring disease caused by this nucleopolyhedrosis virus (NPV) is often referred to as "wilt" due to the soft, limp appearance of the diseased larvae (Figure 2). The disease can reach epizootic (= outbreak) proportions as gypsy moth population densities increase (Doane 1970). These epizootics result from increased transmission rates, within and between generations of the gypsy moth, as small larvae become infected and die on leaves in the crowns of trees. These larval cadavers disintegrate and serve as inocula for healthy feeding larvae. Also, virus transmission occurs when adult females deposit their egg masses on NPV-contaminated surfaces (*transovum* transmission); larvae hatching from these contaminated eggs have a high risk of contracting the disease in the following spring. Virus infection is probably initiated at low host densities and as the host density increases the virus spreads due to density-dependent processes (Woods and Elkinton 1987). Birds and mammals have the ability to pass and disperse active gypsy moth NPV (Lautenschlager and Podgwaite 1979) and parasites and invertebrate predators may play a role in the transmission of gypsy moth NPV within natural populations. Reardon and Podgwaite (1976) found significant positive correlations between the incidence of NPV disease and the



Figure 2.Gypsy Moth Larvae Infected with the Nucleopolyhedrosis Virus

incidences of the parasites *Cotesia melanoscelus* and *Parasetigena agilis* and further, Raimo et al. (1977), showed that *Cotesia* females could transmit NPV from infected to healthy gypsy moth larvae. In many dense gypsy moth populations, the virus kills up to 90 percent of the larvae and reduces populations to levels where they cause only minimal defoliation and tree damage in the following year.

In the late 1950's, the USDA Forest Service began to explore the feasibility of developing these naturally occurring diseases as

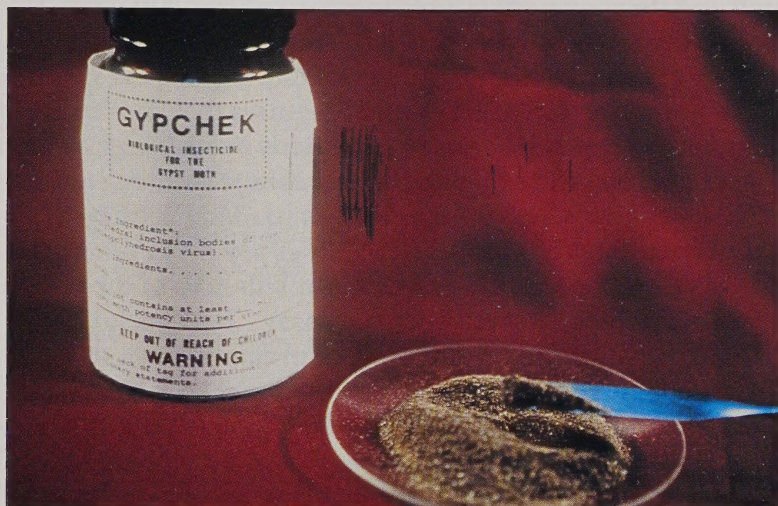


Figure 3. The Gypsy Moth Nucleopolyhedrosis Virus Product, GYPCHEK

alternatives to chemical insecticides for suppressing gypsy moth populations. In April 1978, following several years of research and development, the gypsy moth nucleopolyhedrosis virus product **GYPCHEK** (Figure 3) was registered by the Environmental Protection Agency (EPA) as a general use pesticide for aerial and ground application to control gypsy moth. Current labelling requires that the product be used under Forest Service supervision. Today, **GYPCHEK** is one of two biological insecticides (the other being *Bacillus thuringiensis kurstaki*) currently registered for use against gypsy moth. The Hamden strain of the gypsy moth NPV (isolated from a Connecticut population) is the active ingredient in the currently registered product.

GYPCHEK has an extremely narrow host range and has no effect on beneficial insects. It has been shown to have no adverse effects on vertebrates including man. The possibility that the gypsy moth NPV may be related to the arthropod-borne (arbo) viruses and other viruses which infect man has been investigated. In studies carried out at Yale University, all of the known arboviruses were found to be serologically unrelated to the NPV of the gypsy moth. Other viruses, including *Herpes* spp., were also found to be serologically unrelated to the gypsy moth NPV (Mazzone et al. 1976).

Since its registration, **GYPCHEK**, has been the subject of intense research and development targeted toward maximizing efficacy. Initial production techniques involved field collecting and processing gypsy moth larvae from populations undergoing NPV epizootics. Current production of **GYPCHEK** requires that it be manufactured under controlled laboratory conditions in a laboratory strain of living gypsy moth larvae (*in vivo*). Field dosage is calculated from insecticidal activity which is expressed as so many activity or potency units per gram of product (GMPU=gypsy moth potency units). A GMPU is the dosage of **GYPCHEK** that kills 50% of the gypsy moth larvae challenged in a laboratory assay. The "early" (prior to 1987) **GYPCHEK** product was a powder prepared from whole, NPV-killed larvae that had been frozen, dehaired and lyophilized. It was applied twice, 7-10 days apart, using conventional aircraft delivery systems. It was tested at various dosages and volumes, and applied in the evening and morning using various types of aircraft and nozzles. The early field dosage was 25 million GMPU or 1×10^{11} polyhedral inclusion bodies (PIB's) per acre per application. Tank mixes contained a sunscreen (to protect the virus from ultraviolet light which rapidly inactivates the virus), a feeding stimulant, and a sticker. Efficacy results from testing the "early" product were often inconsistent, due in part, to the physical nature of the product, low activity, marginal dosages, inadequate sunscreens in the tank mix, or poor timing of application.

In 1987, an "improved" **GYPCHEK** tank mix (Table 1) was evaluated in northern and eastern Maryland as part of the Maryland IPM Gypsy Moth Demonstration Project (Reardon et al 1987). The improved tank mix contained fewer inerts (to eliminate nozzle clogging), more active ingredient (50 million GMPU per gallon), and an effective sunscreen, Orzan LS (a lignosulfonate), to prolong virus

Table 1. Gypchek Tank Mix Ingredients

Ingredient	Amt. per 3.9 liters (1 gal.)
Orzan LS ¹ (ITT Rayonier, Inc., Seattle, WA)	227 g (6% w/v)
Pro Mo liquid supplement ² (Southern States Cooperative, Inc., Richmond, VA)	470 ml (12.5% v/v)
Rhoplex B60A ³ (Rohm & Hass Co., Philadelphia, PA)	77.6 ml (2% v/v)
Stream Water (pH 5.0 - 8.0) Gypchek ⁴	3.24 liters (85% v/v) 7.2 g

¹ Lignosulfonate powder.

² mixture of condensed molasses and corn extracts.

³ aqueous acrylic emulsion.

⁴ lot 226, 3.47×10^{10} polyhedral inclusion bodies/g.

activity on foliage. Results of these tests were encouraging with greater than 95 percent net reduction in egg masses (Podgwaite et al 1987, Webb et al 1989). This tank mix was also evaluated on similar population densities occurring in the mountainous terrain of the George Washington National Forest in Virginia (Figure 4). Results were again positive with a greater than 95 percent net egg mass reduction in 5 of 6 treated plots. Defoliation in the control plots averaged 67 percent as compared to only 22 percent in the **GYPCHEK**-treated plots (Podgwaite and Reardon 1988). Several factors contributed to the effectiveness of the 1987 and 1988 **GYPCHEK** treatments: 1) the tank mix itself and its attributes as previously described; and 2) two applications three days apart allowed more active NPV to be continuously available to the target insect for a five to six day period.

The necessity for two applications (three days apart) at 2 gals/acre (= standard), coupled with a limited supply of product in the face of an increased demand, led to a series of field studies designed to maximize efficacy while minimizing application costs. From 1989 to 1991, as part of the Appalachian IPM Gypsy Moth Project (Reardon



Figure 4. Aerial Application of GYPCHEK to Control Gypsy Moth Populations on the George Washington National Forest in Virginia

1991), several field studies were conducted in Pennsylvania and Virginia to evaluate reduced dosages, reduced volumes and one application versus two applications. The results reaffirmed the superiority of the standard, but also revealed that a lower **GYPCHEK** dosage in a lower emitted volume could protect foliage and reduce egg mass populations.

The "improved" tank mix is efficacious and ready for operational use when applied at 2 gals/acre and high dose (5×10^9 PIB/A/appl). It is recommended that two applications be made (three days apart) against moderate to high density populations (300-5,000 egg masses/acre). To date, there has been only one evaluation of an aerial application of **GYPCHEK** to low density populations (less than 100 egg masses/acre). This evaluation was conducted on the George Washington National Forest in 1989 and the resultant 92 percent net egg mass reduction was encouraging. However, further field tests are necessary before **GYPCHEK** can be considered operational at low population densities.

GYPCHEK should be applied when gypsy moth larvae are small (first and second instars are most susceptible) and since **GYPCHEK** must be ingested to be effective, leaf expansion should be at the maximum (e.g., at least 25% expanded white oak) that is consistent with larval development. The larvae ingest the viral polyhedral

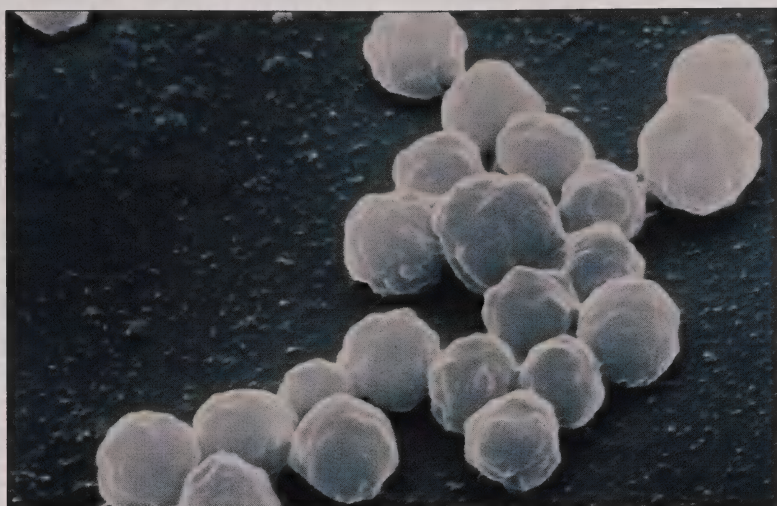


Figure 5. Polyhedral Inclusion Bodies of the Gypsy Moth Nucleopolyhedrovirus Under Magnification

inclusion bodies (PIB's) (Figure 5) along with the foliage, and the rod-shaped virus particles (virions) are liberated as the polyhedral protein matrix dissolves in the gut. The virions invade through the gut wall and attack internal tissues and organs of the larvae, eventually causing a general viral infection. The virus multiplies rapidly in the nucleus of cells of the larvae, eventually causing disintegration of the internal tissues and death. The entire process takes 10 to 14 days, depending upon the size of the larvae, virus dose, and ambient temperature. Larvae about to die have a characteristic oily-shiny appearance. Virus-killed larvae typically hang in an inverted-V, turn brownish-black and are very fragile to the touch. Larval cadavers often rupture releasing a brownish-black liquid that contains large numbers of the polyhedra. Larvae killed by the fungus, *Entomophaga maimaiga* have a similar appearance except that they retain their normal color and are not fragile to the touch. The addition of NPV to the environment at dosages consistent with those used for control of the gypsy moth does not raise NPV levels above those that would occur naturally. Gypsy moth NPV persists naturally at high levels in soil, litter and on bark for at least one year following natural outbreaks (Podgwaite et al. 1979).

Widespread operational use of GYPCHEK hinges on availability and cost. GYPCHEK is not in commercial production, but produced by a

USDA Animal and Plant Health Inspection Service/Forest Service cooperative project yielding 5,000-7,000 acre equivalents (AE) annually at a cost of \$20/AE. Tank mix ingredients add another \$2.00/AE. In general, it takes about 500 to 1,000 infected larvae to produce enough **GYPCHEK** to treat one acre twice.

The recent successful field trials with **GYPCHEK** and the continued spread of the gypsy moth to the south and west, and the environmental concerns over the effects of both *Bt* and Dimilin (the insect growth regulator) on selected non-target organisms have stimulated commercial interests. Recently, several companies have attempted to phase **GYPCHEK** into commercial production using *in vivo* production. Unfortunately, their attempts have been unsuccessful although another large United States-based company which currently produces numerous insecticides for agricultural use is extremely interested. This same company is also continuing efforts for production of **GYPCHEK** using cell culture (*in vitro*) systems.

Also, additional strains of the virus (e.g., Abington strain developed by the Agricultural Research Service) have been identified and the search is continuing for more virulent strains. At the present time, there are no data that indicate that any recently identified strains are more efficacious in the field than the strain (Hamden) used in the current **GYPCHEK** product.

Future field efforts will focus on the evaluation of **GYPCHEK** against low density populations (less than 100 EM/acre) as a component tactic in an integrated pest management (IPM) approach to slow the spread of the gypsy moth. Also, field evaluations will continue in an effort to lower the dosage, volume and numbers of applications of the current formulation. Research will continue toward increasing activity of the product through the substitution of either more virulent natural strains of the virus or genetically engineered viruses. Also, a ready-to-use formulation that extends NPV activity on foliage will be sought.

For additional information concerning **GYPCHEK**, contact either Dr. John Podgwaite - Hamden, CT at (203)773-2033, Dr. Richard Reardon - Morgantown, WV at (304)285-1566, or local Cooperative Extension Service office.

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Pesticide Precautionary Statement

This publication reports the aerial application of insecticides. It does not contain recommendations for insecticide use, nor does it imply that the uses discussed here have been registered. All uses of insecticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

Caution: Insecticides may be injurious to humans, domestic animals, desirable plants, and fish or other wildlife if they are not handled or applied properly. Use all insecticides selectively and carefully. Follow recommended practices for the disposal of surplus insecticides and insecticide containers.

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